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(71) Applicant *(for all designated States except US)*: BIOCON INDIA LIMITED [IN/IN]; 20th K.M. Hosur Road, Hebbagodi, Bangalore District, Bangalore 561 229 (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SIRCAR, Anindya [IN/IN]; BB–3, Salt Lake City, Sector I, Calcutta 700 064 (IN). KHEDKAR, Anand [IN/IN]; 2/2, Skyscraper A, Warden Road, Mumbai 400 026 (IN). KULKARNI, Madhav [IN/IN]; "Bhalachandra", District–Satara, Vaduj 415 506 (IN). SURYANARAYAN, Shrikumar [IN/IN]; 553 B, 8th Main Road, Kormangala, Bangalore 560 034 (IN). SRIDHARAN, Madhavan [IN/IN]; D1, Srinivas Residency, K.R. Gardens, Murugeshpalaya, Bangalore 560 017 (IN). ACHARAYA, Poorpanapranja [IN/IN]; 1002, 12th A Cross, 35th Main, J.P. Nagar, Phase I, Bangalore 560 078 (IN). SAMVASIVAM, Ganesh [IN/IN]; B–3, Kudermukh Colony, Kormangala, Bangalore 560 034 (IN).

(74) Common Representative: BIOCON INDIA LIMITED; Sircar, Anindya, 20th K.M. Hosur Road, Hebbagodi, Bangalore 561 229 (IN).

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(54) Title: METHODS OF PRODUCING ESTERS OF MYCOPHENOLATE

(57) Abstract

Methods for the manufacture of Mycophenolate are disclosed. Mycophenolate mofetil is biochemically synthesized using Mycophenolic Acid and 2–morpholino ethanol with the help of an enzyme. Mycophenolate mofetil is also chemically synthesized non–catalytically by refluxing mycophenolic acid with 2–morpholino ethanol in the absence of a third solvent or a catalyst.

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Methods of producing esters of Mycophenolate.

This invention relates to an improved process for the manufacture of Mycophenolate Mofetil by a biochemical method using enzymes or chemically without the use of any catalyst

BACKGROUND

Mycophenolate mofetil of formula I is the morpholinoethyl ester of Mycophenolic acid (MPA).

Formula I

Mycophenolate mofetil is an immunosuppressant. It is derived from mycophenolic acid which was isolated from a fungus and chemically modified to improve oral absorption. Mycophenolate mofetil, the pharmaceutically acceptable salt thereof is used as an immunosuppressive agent, anti-inflammatory, anti-tumor and anti-viral agent.

Chemical synthesis route for the manufacture of Mycophenolate mofetil already exists. An acid halide condensation route for the synthesizing the Mycophenolate mofetil has been described in US 4,753,935; which requires two steps and has a high dimeric impurity. Those skilled in the field of esterification reactions will appreciate that the conventional teachings for the synthesis of an ester through the reaction of an acid and an alcohol require the use of a chemical catalyst to achieve acceptable yields. The direct

esterification of mycophenolic acid without any catalyst too has been disclosed in US 5,247,083, in which the reaction is carried out in the presence of an inert organic solvent.

It has surprisingly been discovered that good yields of mycophenolate mofetil can be obtained without the disadvantage of prior described methods, without the use of a third organic solvent and without the use of chemical catalysts. It has also been found that it is possible to produce mycophenolate mofetil under very mild conditions using enzymes, in the presence of water and organic solvents and no other chemical catalysts. These processes reduce the chances of unwanted side reaction and lead to purer products.

Summary of the invention.

The present invention concerns methods for making Mycophenolate mofetil by :

(i) reacting the Mycophenolic acid and a molar excess of 2-morpholino ethanol in an organic solvent along with an enzyme and an appropriate quantity of water .

(ii) refluxing Mycophenolic acid with a large excess of 2-morpholino ethanol in the absence of any other organic solvent or a catalyst.

Detailed description of the invention.

This invention relates to a process for the conversion of Mycophenolic acid and 2-morpholino ethanol into Mycophenolate Mofetil.

According to one method of this invention the substrates, MPA and 2-morpholino ethanol are added in an organic solvent or a mixture of more than one organic solvents , water is added to the system to adjust the water content and pH in the microenvironment, enzyme is added to this system, the mixture is incubated at a temperature between 20 to 55 deg C, the reaction is carried out for a time upto 120 hr, the esterified product is analyzed by HPLC method.

The MPA is used in a concentration range of 0.03 to 5%. The 2-morpholino ethanol is used in molar equivalent of 1 to 15 with respect to MPA. The MPA and 2-morpholino ethanol are added to the organic solvent or mixture of organic solvents more than one, where the organic solvent is a C6-C12 alkane such as iso-octane, n-hexane, cyclohexane, heptane, octane or a C2-C12 alcohol such as ethanol, propanol, 2-propanol, hexanol, octanol, or isopropanol. A surfactant is added to the organic solvent or in the mixture of the organic solvents which is Sodium bis (ethylhexyl) sulfosuccinate (Aerosol OT or AOT), Cetyl trimethyl ammonium bromide or Trimethyl octyl ammonium chloride (TOMAC). The water content (Wo), which is the molar ratio of the water to the surfactant, is adjusted to a value in the range of 1 to 30. The pH of the microenvironment is adjusted in a range of 3 to 8 using buffer such as acetate or phosphate buffer. The enzyme, which is used for the bioconversion is a hydrolase which may be lipase, cutinase, esterase or a protease from microbial, animal or plant origin. The enzyme is added in organic solvent in absence or presence of a surfactant. The reaction is carried out at a temperature in a range of 20 to 55 deg C. The time period of reaction is upto 120 hrs. The esterified product is analyzed by HPLC method.

Another method for producing mycophenolate mofetil comprises heating and or refluxing MPA (mycophenolic acid) with a large excess of 2-morpholino ethanol in the absence of any other organic solvent or catalyst. The MPA is heated and optionally refluxed with a large excess of 2-morpholino ethanol at a temperature between 80 to 150 deg C. The reaction is carried out for a time period of 12 to 120 hrs.

Both of these methods are illustrated with examples below which are not intended to be limiting.

Example 1

A 50 mM solution of AOT in 10 ml isooctane was prepared. In the surfactant solution MPA in a concentration of 0.6 mM and 2-morpholino ethanol 0.9 mM were added. To this mixture acetate buffer (pH 5.0) was added to adjust the Wo to 3.0. Lipase from *Candida rugosa* was added in a concentration of 1 mg/ml. The reaction mixture was incubated at a temperature of 37 deg C for 24 hrs. The esterified product was analyzed by HPLC.

Example 2

A 100 mM solution of AOT in 10 ml isooctane was prepared. In the surfactant solution MPA in a concentration of 0.6 mM and 2-morpholino ethanol 9.0 mM were added. To this mixture acetate buffer (pH 4.5) was added to adjust the Wo to 2.8. Lipase from *Mucor meihei* was added in a concentration of 10 mg/ml. The reaction mixture was incubated at a temperature of 37 deg C for 48 hrs. The esterified product was analyzed by HPLC.

Example 3

A 100 mM solution of AOT in 50 ml isooctane was prepared. In the surfactant solution MPA in a concentration of 0.6 mM and 2-morpholino ethanol 9.0 mM were added. To this mixture acetate buffer (pH 4.5) was added to adjust the Wo to 10. Lipase from *Candida albicans* was added in a concentration of 7 mg/ml. The reaction mixture was incubated at a temperature of 37 deg C for 48 hrs. The esterified product was analyzed by HPLC.

Example 4

A 100 mM solution of CTAB in 50 ml isooctane with ethanol as a cosolvent was prepared. In the surfactant solution MPA in a concentration of 0.6 mM and 2-morpholino ethanol 9.0 mM were added. To this mixture phosphate buffer (pH 7.0) was added to adjust the Wo to 20. Pig liver esterase was added in a concentration of 5 mg/ml. The reaction mixture was incubated at a temperature of 37 deg C for 96 hrs. The esterified product was analyzed by HPLC.

Example 5

A 100 mM solution of TOMAC in 50 ml octanol with propanol as a cosolvent was prepared. In the surfactant solution MPA in a concentration of 0.6 mM and 2-morpholino ethanol 9.0 mM were added. To this mixture acetate buffer (pH 4.5) was added to adjust the Wo to 2.8. Protease from serratia marcesens was added in a concentration of 7 mg/ml. The reaction mixture was incubated at a temperature of 45 deg C for 120 hrs. The esterified product was analyzed by HPLC.

Example 6

A microemulsion system using hexane, water and 2 propanol in mole fraction ratio of 0.23:0.32:0.45 was prepared. In the solution MPA in a concentration of 0.6 mM and 2-morpholino ethanol 9.0 mM were added. Protease from *Bacillus subtilis* was added in a concentration of 7 mg/ml. Cutinase was added in a concentration of 7 mg/ml. The reaction mixture was incubated at a temperature of 37 deg C for 48 hrs. The esterified product was analyzed by HPLC.

Example 7

A 150 mM solution of AOT in 100 ml isooctane was added. In the surfactant solution MPA in a concentration of 0.6 mM and 2-morpholino ethanol 9.0 mM were added. To this mixture acetate buffer (pH 4.5) was added to adjust the Wo to 1.5. Lipase from *Mucor meihei* was added in a concentration of 5 mg/ml. The reaction mixture was incubated at a temperature of 20 deg C for 48 hrs. The esterified product was analyzed by HPLC.

Example 8

10mg of MPA was taken in 10mL of 2-morpholino ethanol and the mixture was heated to 100 deg C. The temperature was maintained between 140 to 150 deg C for about 6 hrs. After the reaction was complete, 100mL of ethyl acetate was added, the organic layers were washed with 3x100mL of water, dried over Na_2SO_4 and the ethyl acetate was removed under reduced pressure to afford the product.

Example 9

10mg of MPA was taken in 10mL of 2-morpholino ethanol and the mixture was heated to 140 deg C under reflux. The temperature was maintained between 140 to 150 deg C for about 6 hrs. After the reaction was complete, 100mL of ethyl acetate was added, the organic layers were washed with 3x100mL of water, dried over Na_2SO_4 and the ethyl acetate was removed under reduced pressure to afford the product, mycophenolate mofetil.

Example 10

12.5 mg of MPA was taken in 20 mL of 2-morpholino ethanol and the mixture was heated to 80 deg C. The temperature was maintained between 80 to 85 deg C for about 96 hrs. After the reaction was complete, 100mL of ethyl acetate was added, the organic layers were washed with 3x100mL of water, dried over Na_2SO_4 and the ethyl acetate was removed under reduced pressure to afford the product.

We claim:

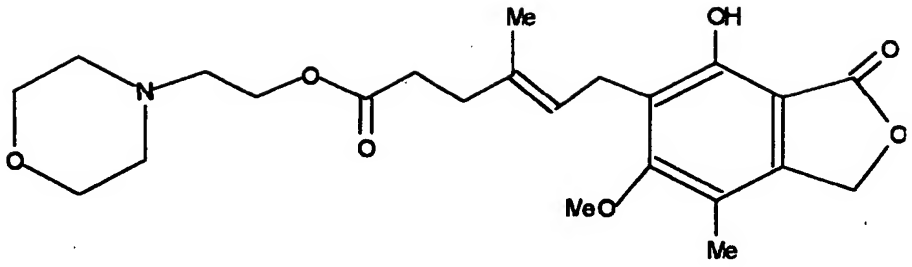
1. A method for the production of mycophenolate mofetil which comprises reacting mycophenolic acid and a molar excess of 2-morpholino ethanol in an organic solvent along with an enzyme and in the presence of water at a temperature between 20 –55 deg C and a pH of 3-8 for a period of upto 120 hrs.
2. The method of claim 1 where the organic solvent is a C6-C12 alkane such as iso-octane, n-hexane, cyclohexane, heptane, octane or a C2-C12 alcohol such as ethanol, propanol, iso propanol, hexanol or octanol.
3. The method of claim 2 where the organic solvent is a mixture of organic solvents .
4. The method of claim 1 where the enzyme catalyst is a hydrolase such as lipase, esterase or protease,
5. The method of claim 1 where the enzyme catalyst is a lipase or esterase.
6. The method of claims 4 and 5 where the enzyme catalyst is an immobilized enzyme .
7. The method of claims 4 and 5 where the enzyme catalyst is in a non-immobilized form.
8. The method of claim 4and 5 where the enzyme is added optionally along with a surfactant .
9. The method of claim 8 where the surfactant is Sodium 2 (ehtylhexyl) sulfosuccinate.
10. The method of claim 8 where the surfactant is Cetyl trimethylammonium bromide.
11. The method of claim 1 where the water content (Wo) is between 1-30, preferably 2-10.

12. A method for the production of mycophenolate mofetil which comprises heating mycophenolic acid with an excess of 2-morpholino ethanol in the absence of any other organic solvent or catalyst.

13. The method of the claim 11, wherein the heating is carried out at a temperature between 80 and 150 degrees.

14. The method of the claim 12 wherein the mixture is refluxed under the said conditions for a time period of 12-120 hrs.

15. The method of the claim 11 to 13, wherein the reaction is carried out for a time period of 12-120 hrs.



Formula I